

PATENT ABSTRACTS OF JAPAN

(11)Publication number : 09-313200

(43)Date of publication of application : 09.12.1997

(51)Int.Cl.

C12Q 1/60

(21)Application number : 08-134727

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(22)Date of filing : 29.05.1996

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(54) DETERMINATION OF LDL CHOLESTEROL

(57)Abstract:

PROBLEM TO BE SOLVED: To easily determine the subject compound without using pretreatment of a specimen by incorporating serum with a specific surfactant and an enzymatic reagent for the determination of cholesterol, reacting cholesterol other than LDL cholesterol and determining the amount of reacted cholesterol.

SOLUTION: Low-density lipoprotein(LDL) cholesterol is easily determined by incorporating serum with a surfactant such as a polyoxyalkylene phenyl ether or a polyoxyethylene alkylene tribenzylphenyl ether, a substance forming a polyanion or a bivalent metal ion exhibiting a bonding affinity to very lowdensity lipoprotein(VLDL) stronger than the affinity to lowdensity lipoprotein(LDL) and an enzymatic reagent for the determination of cholesterol, preferentially reacting the cholesterol in the high-density lipoprotein(HDL) and in the very low-density lipoprotein(VLDL) among the lipoproteins and determining the reaction amount of cholesterol after the preferential reaction.

LEGAL STATUS

[Date of request for examination]

14.03.2001

[Date of sending the examiner's decision of rejection]

[Kind of final disposal of application other than the examiner's decision of rejection or

application converted registration]

[Date of final disposal for application]

[Patent number] 3193634

[Date of registration] 25.05.2001

[Number of appeal against examiner's
decision of rejection]

[Date of requesting appeal against examiner's
decision of rejection]

[Date of extinction of right]

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CLAIMS

[Claim(s)]

[Claim 1] The quantum approach of the low-density lipoprotein cholesterol characterized by measuring the reacting weight of subsequent cholesterol after adding the enzyme reagent for cholesterol measurement to a blood serum in the surfactant chosen from polyoxyethylene alkylene phenyl ether and polyoxyethylene alkylene tribenzyl phenyl ether, and a list and making the cholesterol in high-density lipoprotein and the quality of very low density lipoprotein react preferentially among lipoproteins.

[Claim 2] The quantum approach of the low-density lipoprotein cholesterol characterize by measure the reacting weight of subsequent cholesterol after add the enzyme reagent for cholesterol measurement in the matter in which strong binding affinity be show [rather than] , and a list and make the cholesterol in high-density lipoprotein and the quality of very low density lipoprotein react preferentially among lipoproteins to low-density lipoprotein to the surfactant choose from polyoxyethylene alkylene phenyl ether and polyoxyethylene alkylene tribenzyl phenyl ether to a blood serum , and the quality of very low density lipoprotein .

[Claim 3] The quantum approach of low-density lipoprotein cholesterol according to claim 2 that the matter in which strong binding affinity is shown [rather than] to low-density lipoprotein to the quality of very low density lipoprotein is matter which generates the poly anion or a divalent metallic ion.

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DETAILED DESCRIPTION

[Detailed Description of the Invention]

[0001]

[Field of the Invention] This invention does not have the need for separation actuation, such as centrifugal separation and electrophoresis, and relates to the approach of carrying out the judgment quantum of the cholesterol in low-density lipoprotein (Low Density Lipoprotein; LDL), and the cholesterol in lipoproteins other than LDL efficiently by simple actuation by few samples.

[0002]

[Description of the Prior Art] Into a blood serum, it combines with apoprotein and lipids, such as cholesterol, form the lipoprotein. A lipoprotein is classified into chylomicron, the quality of very low density lipoprotein (Very Low Density Lipoprotein; VLDL), low-density lipoprotein (Low Density Lipoprotein; LDL), high-density lipoprotein (High Density Lipoprotein; HDL), etc. according to the difference in physical description. It is known among these lipoproteins that LDL is one of the causative agents which cause arteriosclerosis.

[0003] Epidemiologically, correlating with the onset frequency of an arteriosclerosis nature disease strongly is proved, and if the measurement of LDL cholesterol of the cholesterol count in LDL is attained by the daily simple approach, it is very useful on clinical.

[0004] The approach of presenting cholesterol measurement as a measuring method of conventional LDL cholesterol, after ultra-centrifugal separation separates LDL with other lipoproteins, for example, and the approach of dyeing a lipid, after electrophoresis separates, and measuring the coloring reinforcement are learned. However, each of these approaches has complicated actuation, or has the problem of being unable to process many specimens, and is hardly used daily. Moreover, sensitization of the antibody combined with lipoproteins other than LDL is carried out to support, the fractionation which was not combined with a sample after mixing is isolated preparatively, and how to measure the cholesterol in the fractionation is also learned. although this approach can be said as the approach which was suitable for everyday measurement as compared with the two previous approaches -- business -- since the process performed by technique was included during measurement actuation, automation was difficult and unsuitable for processing of an a large number specimen too.

[0005] As an approach of carrying out the judgment quantum of the lipoprotein in a sample, without on the other hand using physical separation means, such as centrifugal separation and electrophoresis, the reactivity of the enzyme (mainly cholesterol oxidase and cholesterol esterase) used for cholesterol measurement over the lipoprotein of HDL and others, i.e., chylomicron, and VLDL and LDL is controlled, and the measuring method which draws and carries out the quantum only of the HDL cholesterol to an enzyme reaction is known. For example, the approach of measuring only HDL cholesterol by using a surfactant and a sugar compound according to JP,7-301636,A is indicated, reactivity with an enzyme is controlled by making lipoproteins other than the lipoprotein which should be measured condense according to JP,6-242110,A, and the method of detecting only the cholesterol in the lipoprotein which is the measuring object is indicated. Although these approaches are very useful from the ability to apply to a automatic analysis machine and automate all processes, they cannot stop at

carrying out the judgment quantum of the lipoproteins other than HDL and HDL to the last, and cannot carry out the judgment quantum of LDL, VLDL, and the chylomicron further. Therefore, with these techniques, it cannot respond to the object of measuring LDL cholesterol, without using a separation means.

[0006] Moreover, in JP,7-280812,A, after making LDL once condense, the cholesterol in other lipoproteins is led to the system which does not participate in the quantum system of LDL, and after dissolving condensation of LDL, the method of performing a quantum is indicated by making LDL cholesterol react. However, in this official report, measurement of LDL cholesterol is not absolutely shown at all the solution over a judgment quantum with LDL, indispensable VLDL, and/or indispensable chylomicron like two patent disclosure official reports described previously. Moreover, since there are many reaction routing counters required for measurement, it cannot apply to a common automatic analyzer, but there is a problem that the utility value is also limited extremely.

[0007] Thus, with the technique known conventionally, the information which suggests about [that it is impossible to measure LDL cholesterol efficiently without performing separation actuation] and its possibility does not exist, either.

[0008]

[Problem(s) to be Solved by the Invention] Therefore, the object of this invention does not have the need for pretreatments, such as centrifugal separation and electrophoresis, can be efficiently measured by simple actuation, and is to offer the judgment assay of LDL cholesterol applicable to various automatic analysis machines.

[0009]

[Means for Solving the Problem] If this invention person etc. performs a reaction with the enzyme reagent for cholesterol measurement in this actual condition under existence of the specific surfactant which dissolves a lipoprotein as a result of inquiring wholeheartedly While the reaction of the cholesterol in HDL and VLDL is accelerated, the reaction of the cholesterol in LDL remarkably Delay, In order that the reaction of the cholesterol in HDL and VLDL may precede the reaction of the cholesterol in LDL and may end By choosing a measure point suitably, judgment measurement of the LDL cholesterol could be carried out, and a header and this invention were completed for moreover it being applicable also to an automatic analyzer.

[0010] That is, after this invention adds the enzyme reagent for cholesterol measurement to a blood serum in the surfactant chosen from polyoxyethylene alkylene phenyl ether and polyoxyethylene alkylene tribenzyl phenyl ether, and a list and makes the cholesterol in HDL and VLDL react preferentially among lipoproteins, it offers the quantum approach of the LDL cholesterol characterized by measuring the reacting weight of subsequent cholesterol.

[0011] moreover , after this invention add the enzyme reagent for cholesterol measurement in the matter in which strong binding affinity be show [rather than] , and a list and make the cholesterol in HDL and VLDL react preferentially among lipoproteins to LDL to the surfactant choose from polyoxyethylene alkylene phenyl ether and polyoxyethylene alkylene tribenzyl phenyl ether to a blood serum , and VLDL , it offer the quantum approach of the LDL cholesterol characterize by measure the reacting weight of subsequent cholesterol .

[0012]

[Embodiment of the Invention] The surfactant chosen from the polyoxyethylene alkylene phenyl ether and polyoxyethylene alkylene tribenzyl phenyl ether which are used by this invention is a surfactant which dissolves a lipoprotein, as a commercial item, emulgen A-60 (Kao Corp. make) etc. is mentioned as a former example, and emulgen B66 (Kao Corp. make) etc. is mentioned as a latter example. This surfactant is independent, or although it can use combining two or more sorts, and the amount used changes with compounds and it is not restricted especially, it is desirable to usually use it by 0.01 - 2% of the weight of concentration for every analysis apparatus which should apply a reagent, so that it may become the sensibility which can detect LDL cholesterol in the desirable measuring time.

[0013] Moreover, when making into a specimen the blood serum with which the measuring method of this invention has the desirable to LDL rather than [as opposed to / further / VLDL] thing to perform

under existence of the matter in which strong binding affinity is shown with a blood serum, and has the chyle especially, a good result will be obtained if it measures by adding the matter concerned. As such matter, the matter which generates the poly anion and a divalent metal salt is mentioned, and, specifically, the chlorides of the divalent metal of $MgCl_2$, $CaCl_2$, $MnCl_2$, and $NiCl_2$ grade, these hydrates, etc. are mentioned as a poly anion as matter with which a tungstophosphoric acid and its salt, dextran sulfate, heparin, etc. generate a divalent metallic ion. These matter is independent, or although it can use combining two or more sorts, and the amount used changes with classes of matter and it is not limited especially, it is desirable to use [in the case of the poly anion] 0.002 to 10% of the weight as reaction final concentration in the range used as 0.01 - 1 % of the weight in the case of the matter which generates a divalent metallic ion.

[0014] Even if it faces adding the matter in which strong binding affinity is shown [rather than] to LDL to a surfactant and VLDL to the blood serum which is a specimen, it adds the enzyme reagent for cholesterol measurement separately in the former and a latter list and it adds separately the mixture of either the former or the latter another side, and the enzyme reagent for cholesterol measurement, three persons may be mixed and you may add as the same reagent.

[0015] Although well-known all of an enzyme-measuring method can be used as a measuring method of cholesterol, the approach of using combining the approach, the cholesterol esterase, and the cholesterol dehydrogenase which are used combining cholesterol esterase and cholesterol oxidase, for example as an enzyme reagent etc. is mentioned. The approach of using combining cholesterol esterase and cholesterol oxidase is [among these] desirable. Moreover, after adding these enzyme reagents for cholesterol measurement, the absorbance analysis which especially the method of detecting cholesterol eventually is not restricted, for example, is performed, combining a peroxidase and a chromogen further, the approach of carrying out direct detection of a coenzyme or the hydrogen peroxide, etc. are mentioned.

[0016] In order to detect the reaction of LDL cholesterol It is necessary to measure the reacting weight after the reaction of the cholesterol in lipoproteins other than LDL ends. After carrying out a fixed time amount reaction and completing mostly the reaction of the cholesterol in lipoproteins other than LDL The approach of acting as the monitor of the advancing reaction dynamically and the approach (two points law) of measuring the reaction which adds a reaction accelerator separately and is produced for the object which promotes the reaction of LDL further by the reaction end point method, and amending with a blank value can be used. The surfactant same as a reaction accelerator used in law 2 point as having used for the reaction of the cholesterol in lipoproteins other than LDL can be used more by high concentration, and also the surfactant of another class can also be used. Moreover, it is also possible to lead cholesterol to another system of reaction which does not participate in the quantum system of the LDL cholesterol in degree process, and to detect only the reaction of LDL cholesterol to the reaction time of the cholesterol in lipoproteins other than LDL, in law, 2 point.

[0017] In addition, although the chylomicron which usually appears only immediately after the ingestion is mentioned as other lipoproteins which exist in a blood serum, that reactivity of this thing is almost the same as that of VLDL. For this reason, since the reactivity of chylomicron as well as VLDL is promoted by addition of the matter which generates the poly anion and a divalent metallic ion and the reaction of chylomicron is also ended at the time of reaction termination of VLDL also when chylomicron exists in a blood serum, the judgment quantum of LDL cholesterol is possible by measuring the reacting weight of subsequent cholesterol.

[0018]

[Effect of the Invention] Since it is widely applicable to the automatic analyzer which there is no need for separation actuation, such as centrifugal separation and electrophoresis, can carry out the judgment quantum of the cholesterol in LDL to the cholesterol in other lipoproteins efficiently by simple actuation, and is used in a clinical laboratory test according to this invention, it is very useful on clinical.

[0019]

[Example] Next, although an example is given and this invention is explained further, this invention is

not limited to these.

[0020] The example 1 right fat blood serum was made into the sample, by this invention approach, LDL cholesterol was measured with the Hitachi 7070 mold automatic analyzer, and the measured value was compared with the measured value obtained by ultracentrifugation. This result is shown in drawing 1. That is, 0.02 % of the weight of tungstophosphoric-acid sodium and 300micro of $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$ reagents 1 containing 0.2 % of the weight were added in 4micro of specimens 1. Next, after about 5 minutes, 100micro of emulgen A-60 (Kao Corp. make) cholesterol measurement reagents 1 containing 0.04 % of the weight of cholesterol esterase 1U/ml, cholesterol oxidase 1U/ml, par oxidase 1U/ml, 0.005 % of the weight [of 4-aminoantipyrines], N, and N-dimethyl meta-toluidines was added, and the absorbance change in 545nm of the after [5 minutes] of the after [1 minute] of the 2nd reagent addition was measured 0.5% of the weight. On the other hand, ultracentrifugation carried out centrifugal [of the 100,000g of the blood serums] with the ultracentrifuge for 2 hours, and removed the upper layer. 1ml was taken from the obtained lower layer, 50micro of heparin (5000usp unit / ml) solution 40microl and MgCl_2 (1M) solutions 1 was added, centrifugal was carried out by 5000 revolutions for 30 minutes, and supernatant liquid was obtained. The fractionation supernatant liquid (HDL is included) which added heparin and MgCl_2 solution to the lower layer solution (LDL and HDL are included) and list which were obtained by ultra-centrifugal separation, and was obtained was given to cholesterol measurement, and the total value of both value was made into the LDL cholesterol value (reference-aul S.Bachorik et al., Clin.Chem.41/10, 1414-1420, 1955). As shown in drawing 1, although the sample to be used could perform this invention approach by simple actuation only, the measured value which has the conventional ultracentrifugation and good functionality was obtained.

[0021] The specimen containing the chyle blood serum which went up to altitude was made into the sample, and by this invention approach, the example 2 triglyceride value measured LDL cholesterol with the Hitachi 7070 mold automatic analyzer, and compared the measured value with the measured value obtained by ultracentrifugation. This result is shown in drawing 2. That is, 300micro of emulgen B66 (Kao Corp. make) reagents 1 containing cholesterol esterase 0.3U/ml, cholesterol oxidase 0.3U/ml, par oxidase 0.3U/ml, and 0.002 % of the weight of 4-aminoantipyrines was added 0.5% of the weight in 4micro of specimens 1. Next, 100micro of Triton X-100 reagents 1 containing 0.04 % of the weight of 1-% of the weight and N, and N-dimethyl meta-toluidines was added after about 5 minutes, the absorbance (it amends in consideration of change of the amount of reagents) in 545nm in front of the 2nd reagent addition was deducted from the absorbance in 545nm 5 minutes after the 2nd reagent addition, and the absorbance change was measured. On the other hand, ultracentrifugation was enforced like the example 1. As shown in drawing 2, the LDL cholesterol measured value by the conventional ultracentrifugation and the measured value which has good functionality were obtained like the example 1.

[0022] In example 3 example 2, except making 0.3 % of the weight of tungstophosphoric acids contain further in the 1st reagent, it was similarly operated using the same specimen and the same reagent, and the measured value by this invention approach was compared with the measured value obtained by ultracentrifugation. This result is shown in drawing 3. As shown in drawing 3, even if it used the blood serum sample containing a chyle blood serum, the LDL cholesterol measured value by the conventional ultracentrifugation and the measured value which has very good functionality were obtained like the example 1.

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